

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 94-105 have been added.

Claims 3-10, 14, 18, 21, 24, 31, 32, 34-36, 38-40, 43, 45-49, 53-54, 58-59, 61, 63-67, 70-75, 77, 79, 80-82, 85, 92, and 93 have been amended as follows:

3 (amended). Method of claim [1 or] 2, comprising the further step of determining the sequence of the tag.

4 (amended). Method of [any of claims 1 to 3] claim 1, comprising the further step of quantifying the tag.

5 (amended). Method of [any of claims 1 to 4] claim 1, wherein the single stranded polynucleotide tag comprises or essentially consists of deoxyribonucleic acid.

6 (amended). Method of [any of claims 1 to 4] claim 1, wherein the single stranded polynucleotide tag comprises only a single polynucleotide strand and no complementary strand, or a part thereof, capable of forming with the single stranded polynucleotide tag a double stranded polynucleotide comprising complementary polynucleotides, including any double stranded polynucleotide wherein at least a part of the double stranded polynucleotide consists of single, complementary polynucleotides.

7 (amended). Method of [any of claims 1 to 4] claim 1, wherein the single stranded polynucleotide tag comprises less than 20 nucleotides.

8 (amended). Method of [any of claims 1 to 4] claim 1, wherein the single stranded polynucleotide tag comprises 10 nucleotides.

9 (amended). Method of claim 7 [or 8], wherein all of said nucleotides of the single stranded polynucleotide tag originate from a cDNA obtained from [the] a biological sample, or from genomic DNA obtained from [the] a biological sample, or from extra-genomic DNA obtained from the biological sample.

10 (amended). Method of [any of claims 1 to 4] claim 1, wherein the cleavage agent capable of recognizing a double stranded polynucleotide comprising complementary polynucleotide strands and cleaving only one of the strands is a site-specific nicking endonuclease.

14 (amended). Method of [any of claims 1 to 13] claim 1 for obtaining at least one single stranded polynucleotide tag from a biological sample, wherein the method comprises, prior to the step of obtaining at least one single stranded polynucleotide tag, the further step of contacting and cleaving

the double stranded polynucleotide provided in step i), or

the double stranded polynucleotide of step ii) contacted and cleaved in one strand by the at least one cleavage agent, preferably a site-specific nicking endonuclease, capable of recognizing a double stranded polynucleotide comprising complementary polynucleotide strands and cleaving only one of the strands of the polynucleotide with at least one cleavage agent, preferably a site-specific restriction endonuclease, capable of recognizing a double stranded polynucleotide comprising complementary polynucleotide strands and cleaving both of the strands of the polynucleotide,

wherein the cleavage of only one strand, or both strands, of the double stranded polynucleotide occurs simultaneously, or sequentially in any order.

18 (amended). Method of [any of claims 1 to 17] claim 6, wherein the method comprises the further step of providing at least one adapter oligonucleotide comprising at least one recognition motif, or a part thereof, for at least one cleavage agent capable of recognizing a double stranded polynucleotide comprising complementary strands and cleaving a) only one complementary strand, or b) both of the complementary strands of the double stranded polynucleotide.

21 (amended). Method of [any of claims 18 to 20] claim 18, wherein the adapter comprises at least two recognition motifs, or a single stranded part thereof, wherein at least

hybridized to the single stranded, first unique nucleotide sequence of a first identifying linker oligonucleotide, said method comprising

contacting at least one or a plurality of said hybrid or chimeric polynucleotide tags[resulting from any of claims 40 to 73]with at least one or a plurality of second identifying linker oligonucleotides,

wherein each second identifying linker oligonucleotide comprises a single stranded, second unique oligonucleotide sequence,

wherein the single stranded, unique second nucleotide sequence of each second identifying linker oligonucleotide comprises essentially all possible combinations of second oligonucleotide sequences, or a subset of such sequences,

wherein each second identifying linker oligonucleotide further comprises at least one molecular identifier and/or at least one selectively detectable label capable of identifying the second identifying linker oligonucleotide,

wherein the contacting of step i) occurs under conditions allowing a hybridization to occur between at least one of the second identifying linker oligonucleotides and at least one hybrid polynucleotide tag, and optionally removing any unhybridized second identifying linker oligonucleotide,

determining the presence and/or amount of any hybridized second identifying linker oligonucleotide comprising a second unique oligonucleotide sequence by means of detection of the label and/or the molecular identifier, and optionally

repeating steps i) and/or ii) until substantially all of the second identifying linker oligonucleotides in the hybridization array, or a predetermined subset thereof, have been tested.

75 (amended). Method of [any of claims 40 to 74] claim 40, wherein any hybridization step is followed by or performed simultaneously with a ligation step.

77 (amended). Method for amplification of a hybrid polynucleotide tag obtainable by [any of claims 40 to 76] claim 40, wherein the method comprises the steps of

obtaining at least one hybrid polynucleotide tag or at least one chimeric polynucleotide tag comprising

a single stranded polynucleotide tag hybridized or ligated to one or both of

a first identifying linker oligonucleotide comprising a single stranded, first unique oligonucleotide sequence, and

a second identifying linker oligonucleotide comprising a single stranded, second unique oligonucleotide sequence

wherein said first identifying linker oligonucleotide and said second [identifying] identifying linker oligonucleotide comprises single stranded nucleotide sequences complementary to at least a part of the nucleotide sequence of the single stranded polynucleotide tag, and

amplifying the at least one hybrid or chimeric polynucleotide tag.

79 (amended). Method for identifying a cDNA in a biological sample, said method comprising the steps of any of the methods for obtaining and characterizing a single stranded polynucleotide tag according to [any of claims 40 to 78] claim 40, said method comprising the further steps of

comparing for at least one of a plurality of predetermined positions in a hybridization array, or for at least one of a plurality of predetermined positions in a capillary tube of a microfluid device,

the sequence of the at least one single stranded polynucleotide tag and/or the amount of the at least one single stranded polynucleotide tag with

the sequence and/or amount of a predetermined polynucleotide tag obtained from a predetermined cDNA, and

identifying a cDNA present in the biological sample.

80 (amended). A method for diagnosing a clinical condition, said method comprising the steps of

determining for at least one of a plurality of predetermined positions in a hybridization array, or for at least one of a plurality of predetermined positions in a capillary tube of a microfluid device, at least one predetermined cDNA in a biological sample by performing a method according to [any of claims 40 to 79] claim 40,

wherein each of the first identifying linker oligonucleotides comprises a predetermined single stranded, first unique oligonucleotide sequence,

wherein each of the second identifying linker oligonucleotides comprises a predetermined single stranded, second unique oligonucleotide sequence,

wherein at least one of said first and second identifying linker oligonucleotides comprises at least one selectively detectable molecular identifier and/or at least one selectively detectable label,

wherein the predetermined cDNA is determined by assaying for a predetermined polynucleotide tag originating from said predetermined cDNA,

wherein the predetermined polynucleotide tag originating from said predetermined cDNA comprises a nucleotide sequence complementary to the sequence of the first and second identifying linker oligonucleotides,

wherein the at least one predetermined position in the hybridization array, or the at least one predetermined position in the capillary tube of a microfluid device, in combination with the determination of the at least one selectively detectable molecular identifier and/or the at least one selectively detectable label comprised by at least one of said first and second identifying linker oligonucleotides, is positively correlated with the presence in the biological sample of the at least one predetermined cDNA, and

diagnosing the clinical condition.

81 (amended). Method of [any of the previous claims] claim 1, wherein at least one cleavage agent is attached to a solid support.

82 (amended). Method of [any previous claim] claim 35, wherein a ligation step is carried out by using a ligase that is attached to a solid support.

83 (amended). Method of [any of claims 81 and 82] claim 81, wherein the solid support is a capillary tube with a diameter of less than 1 mm.

84 (amended). Method of claim 83, wherein the solid support is a [capilaty] capillary tube with a diameter less than 0,1 mm.

85 (amended). Method of claim[s] 81, wherein the solid support forms part of the inside of a chamber of a microfluid device.

92 (amended). [The] A solid support [according to claim 9], comprising a plurality of ordered first identifying linker oligonucleotides, or a subset of such oligonucleotides, wherein at least one of said first identifying linker oligonucleotides comprises a single stranded nucleotide sequence hybridized to at least single stranded polynucleotide tag comprising a sequence complementary thereto wherein the single stranded poly-nucleotide tag is obtained by a method of [any of claims 1 to 85] claim 24.

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93 (amended). The solid support according to claim 91, wherein the single stranded poly-nucleotide tag is obtained by displacement of a double stranded polynucleotide tag comprising at least partly complementary nucleotide strands.

61 (amended). Method of [any of claims 50 to 60] claim 50, wherein substantially each chimeric polynucleotide tag fragment is ligated to the second identifying linker oligonucleotide hybridized thereto.

63 (amended). Method of [any of claims 50 to 62] claim 50, wherein substantially each of the plurality or subset of second identifying linker oligonucleotides further comprises a molecular identifier capable of characterizing and/or separating the linker oligonucleotides and/or hybrid oligonucleotide tags according to i) the molecular weight and/or ii) charge and/or iii) an electromagnetic property and/or iv) an ability to emit electromagnetic radiation after excitation of individual linker oligonucleotides comprising individual molecular identifiers.

64 (amended). Method of [any of claims 50 to 63] claim 50, wherein substantially each of the plurality or subset of second identifying linker oligonucleotides further comprises a selectively detectable label capable of identifying substantially individual identifying linker oligonucleotides and/or hybrid oligonucleotide tags and/or chimeric oligonucleotide tags forming part of a plurality of such oligonucleotides, or a subset thereof.

65 (amended). Method of [any of claim 50 to 64] claim 50, wherein the maximum number of combinations of single stranded second unique nucleotide sequences is 4^n , wherein n denotes the number of nucleotides in the unique nucleotide sequence.

66 (amended). Method of [any of claims 50 to 65] claim 50, wherein each sample comprising the at least one single stranded polynucleotide tag is located in a separate container.

67 (amended). Method of [any of claims 40 to 66] claim 43 for determining the sequence of a part of a single stranded polynucleotide tag hybridized or ligated to an identifying linker oligonucleotide, said method comprising the further steps of

i) contacting

a) a solid support comprising a hybridization array comprising an ordered plurality of first identifying linker oligonucleotides comprising a single stranded first unique oligonucleotide sequence, with

b) a sample comprising at least one single stranded polynucleotide tag, or a plurality of samples obtained by dividing a composition comprising a plurality of single stranded polynucleotide tags into[at least about 4 samples, for example] at least about 16 samples[, [such as at least about 256 samples, for example at least about 1024 samples, such as at least about 4096 samples],

wherein each set of first identifying linker oligonucleotides comprising a single stranded first unique oligonucleotide sequence is identifiable by their location in the hybridization array,

wherein essentially all possible combinations of single stranded first unique nucleotide sequences of first identifying linker oligonucleotides, or a subset of such combinations, are represented in the array,

wherein at least one single stranded polynucleotide tag comprised in the sample is hybridized to a complementary single stranded first unique nucleotide sequences of a first identifying linker oligonucleotide,

wherein the hybridization of the at least one single stranded polynucleotide tag to a complementary single stranded first unique nucleotide sequence occurs at an identifiable position in the hybridization array,

wherein said hybridization generates a hybrid nucleotide tag comprising the at least one single stranded polynucleotide tag hybridized to a complementary single stranded first unique nucleotide sequence of a first identifying linker oligonucleotide, and optionally

ii) determining the position in the hybridization array of the hybrid polynucleotide tag, by

iii) correlating the position in the hybridization array of the hybrid polynucleotide tag with the corresponding single stranded first unique nucleotide sequence, and

iv) determining the sequence of the part of the single stranded polynucleotide tag that is hybridized to the complementary single stranded first unique nucleotide sequence at the determined position in the hybridization array.

70 (amended). Method of [any of claims 67 to 69] claim 67, wherein substantially each of the plurality or subset of first identifying linker oligonucleotides further comprises a molecular identifier capable of characterizing and/or separating the linker oligonucleotides and/or hybrid oligonucleotide tags and/or chimeric oligonucleotide tags according to i) the molecular weight and/or ii) charge and/or iii) an electromagnetic property and/or iv) an ability to emit electromagnetic radiation after excitation of individual linker oligonucleotides comprising individual molecular identifiers.

71 (amended). Method of [any of claims 67 to 70] claim 67, wherein substantially each of the plurality or subset of first identifying linker oligonucleotides further comprises a selectively detectable label capable of identifying substantially individual identifying linker oligonucleotides and/or hybrid oligonucleotide tags and/or chimeric oligonucleotides forming part of a plurality of such oligonucleotides, or a subset thereof.

72 (amended). Method of [any of claims 67 to 71] claim 67, wherein the maximum number of combinations of single stranded first unique nucleotide sequences is 4^n , wherein n denotes the number of nucleotides in the unique nucleotide sequence.

73 (amended). Method of [any of claims 67 to 72] claim 67, wherein each sample comprising the at least one single stranded polynucleotide tag is located in a separate container.

74 (amended). Method of [any of claims 40 to 73] claim 43, wherein the method comprises the further steps of determining at least part of the sequence of the tag not

one of said motifs are capable of binding a site-specific nicking endonuclease capable of recognizing a double stranded polynucleotide comprising complementary strands and cleaving only one complementary strand thereof.

24 (amended). Method of [any of claims 18 to 23] claim 18 for obtaining at least one single stranded polynucleotide tag from a biological sample, said method comprising the steps of

providing at least one adapter oligonucleotide comprising

at least one recognition motif for at least one site-specific nicking endonuclease, wherein said motif comprises a double stranded polynucleotide comprising complementary polynucleotide strands, or

a part of a recognition motif for at least one site-specific nicking endonuclease, wherein said part comprises a single polynucleotide strand which, together with a complementary polynucleotide strand, forms a recognition motif for at least one site-specific nicking endonuclease,

further providing

at least one ribonucleic acid obtained from the biological sample, or

at least one double stranded polynucleotide fragment comprising complementary polynucleotide strands, wherein said double stranded polynucleotide is obtained by a method comprising the step of using the at least one ribonucleic acid provided in step iic) as a template for the synthesis of a polynucleotide strand complementary to the at least one ribonucleic acid, or

at least one double stranded genomic polynucleotide fragment, or at least one double stranded extra-genomic polynucleotide fragment, wherein said genomic polynucleotide fragment or extra-genomic polynucleotide fragment is obtained by cleaving a genomic polynucleotide or an extra-genomic polynucleotide with at least one site-specific restriction endonuclease

capable of recognizing a double stranded polynucleotide comprising complementary strands and cleaving both of said strands,

obtaining a double stranded chimeric polynucleotide comprising an adapter oligonucleotide part by

iiia) linking together

the at least one adapter oligonucleotide of step ia) comprising the at least one recognition motif for the at least one site-specific nicking endonuclease, wherein said motif comprises complementary strands, with either

the at least one double stranded polynucleotide comprising complementary polynucleotide strands, wherein said double stranded polynucleotide is obtained by a method comprising the step of using the at least one ribonucleic acid provided in step iic) as a template for the synthesis of a polynucleotide strand complementary to the at least one ribonucleic acid, or

the at least one double stranded genomic polynucleotide or the at least one double stranded extra-genomic polynucleotide of step iie),

or

iiib) obtaining a double stranded chimeric polynucleotide comprising an adapter oligonucleotide part by linking together

at least one adapter oligonucleotide comprising a part of a recognition motif for at least one site-specific nicking endonuclease, wherein said part comprises a single oligonucleotide strand which, together with a complementary strand, forms a recognition motif for at least one site-specific nicking endonuclease,

with

the at least one ribonucleic acid obtained from the biological sample,

and

obtaining at least one double stranded chimeric polynucleotide comprising an adapter oligonucleotide part by using the chimeric polynucleotide obtained by linking together the adapter oligonucleotide of step iiibi) with the ribonucleic acid of step iiibj) as a template for the synthesis of a polynucleotide strand complementary to said chimeric polynucleotide,

contacting and cleaving the double stranded chimeric polynucleotide obtained in step iiia) or step iiib) with either

iva) at least one site-specific nicking endonuclease capable of recognizing a double stranded polynucleotide comprising complementary polynucleotide strands and cleaving only one of said strands,

or contacting and cleaving the double stranded chimeric polynucleotide obtained in step iiia) or step iiib) with

ivb) a combination of

at least one site-specific restriction endonuclease capable of recognizing a double stranded polynucleotide comprising complementary strands and cleaving both of said strands, and

b) at least one site-specific nicking endonuclease capable of recognizing a double stranded polynucleotide comprising complementary polynucleotide strands and cleaving only one of said strands,

wherein the contacting and cleaving of the double stranded chimeric polynucleotide performed with the combination of step ivb) occurs either simultaneously, or sequentially in any order, and

obtaining at least one single stranded polynucleotide tag.

31 (amended). Method of [any of claims 24 to 28] claim 24, wherein the ribonucleic acid comprises mRNA.

32 (amended). Method of claim 31, wherein the ribonucleic acid comprises mRNA that is polyadenylated.

34 (amended). Method of [any of the previous claims] claim 24, wherein the site-specific restriction endonuclease capable of recognizing complementary strands of a double stranded polynucleotide recognizes a motif comprising [8 nucleotides, or less than 8 nucleotides, such as 7 nucleotides, or] less than 7 nucleotides[, such as 6 nucleotides, or less than 6 nucleotides, such as 5 nucleotides, or less than 5 nucleotides, such as 4 nucleotides].

35 (amended). Method of [any of claims 24 to 30] claim 24, wherein the chimeric polynucleotide is obtained by means of ligation.

36 (amended). Method of [any of claims 24 to 30] claim 24 comprising the further step of contacting the double stranded polynucleotide with a site-specific methylase or methyltransferase.

38 (amended). Method of [any of claims 24 to 28] claim 24, wherein a methylated dCTP analog is substituted for an unmodified dCTP in the synthesis reaction resulting in the synthesis of a complementary strand to the template.

39 (amended). Method of [any of the previous claims] claim 1 comprising the further step of [(i) characterizing, ii)] separating [and/or iii) identifying and/or iv) determining the amount of] the at least one single stranded polynucleotide tag from other single stranded polynucleotides and/or double stranded polynucleotides.

40 (amended). Method of claim 39 comprising the further steps of [characterizing and/or identifying and/or] separating the at least one single stranded polynucleotide tag by

forming a hybrid polynucleotide tag and/or a chimeric polynucleotide tag between at least one single stranded polynucleotide tag and a complementary, single stranded first unique nucleotide sequence of a first identifying linker oligonucleotide, said method comprising the steps of

- i) providing a sample preferably comprising at least one single stranded polynucleotide tag, or a plurality of samples obtained by dividing a composition comprising a plurality of single stranded polynucleotide tags into [at least about 4 samples, for example] at least about 16 samples, [such as at least about 256 samples, for example at least about 1024 samples, such as at least about 4096 samples,]
- ii) contacting each of the plurality of samples, or a subset thereof, provided in step i) with at least one first identifying linker oligonucleotide, or a plurality of first identifying linker oligonucleotides,

wherein each first identifying linker oligonucleotide comprises a single stranded first unique nucleotide sequence,

wherein the at least one single stranded polynucleotide tag, or each of the plurality of single stranded polynucleotide tags, or a subset thereof, in each of the samples is contacted with essentially only one first identifying linker oligonucleotide comprising a single stranded first unique nucleotide sequence,

wherein preferably each sample is contacted with essentially all possible combinations of single stranded first unique nucleotide sequences of the first identifying linker oligonucleotide, or a predetermined subset of such combinations,

wherein at least one single stranded polynucleotide tag in each sample comprises a polynucleotide sequence, or a part thereof, complementary to a single stranded first unique nucleotide sequence of at least one first identifying linker oligonucleotide contacting the sample,

wherein the contacting of each of the plurality of samples, or a subset thereof provided in step i), with at least one or a plurality of first identifying linker oligonucleotides, occurs under conditions allowing a hybridization to occur between

at least one first identifying linker oligonucleotide comprising a single stranded first unique nucleotide sequence, and

at least one single stranded polynucleotide tag complementary to the single stranded first unique nucleotide sequence, and optionally

removing by means of one or more washing steps any unhybridized material from the hybrid polynucleotide tags and/or the chimeric polynucleotide tags formed between the single stranded polynucleotide tag and the complementary, single stranded first unique nucleotide sequence of the first identifying linker oligonucleotide.

43 (amended). Method of [any of claims 40 to 42] claim 40, wherein substantially each tag is ligated to the first identifying linker oligonucleotide hybridized thereto.

45 (amended). Method of [any of claims 40 to 44] claim 40, wherein substantially each of the plurality or subset of first identifying linker oligonucleotides further comprises a molecular identifier capable of characterizing and/or separating the linker oligonucleotides and/or hybrid oligonucleotide tags according to i) the molecular weight and/or ii) charge and/or iii) an electromagnetic property and/or iv) an ability to emit electromagnetic radiation after excitation of individual linker oligonucleotides comprising individual molecular identifiers.

46 (amended). Method of [any of claims 40 to 45] claim 40, wherein substantially each of the plurality or subset of first identifying linker oligonucleotides further comprises a selectively detectable label capable of identifying substantially individual identifying linker oligonucleotides and/or hybrid oligonucleotide tags forming part of a plurality of such oligonucleotides, or a subset thereof.

47 (amended). Method of [any of claim 40 to 46] claim 40, wherein the maximum number of combinations of single stranded first unique nucleotide sequences is 4^n , wherein n denotes the number of nucleotides in the unique nucleotide sequence.

48 (amended). Method of [any of claims 40 to 47] claim 40, wherein each sample comprising the at least one single stranded polynucleotide tag is located in a separate container.

49 (amended). Method of [any of claims 40 to 48] claim 40, wherein the at least one or a plurality of first identifying linker oligonucleotides comprises a recognition motif for a site-specific restriction endonuclease, wherein the recognition motif is correlated to the sequence of nucleotides in the single stranded first, unique nucleotide sequence.

53 (amended). Method of [any of claims 50 to 52] claim 50 and comprising the further step of contacting the at least one or a plurality of chimeric polynucleotide tags with a site-specific nicking endonuclease capable of recognising a recognition motif of the chimeric polynucleotide tag fragment and cleaving a single strand of said fragment and providing a single stranded polynucleotide tag.

54 (amended). Method of [any of claims 40 to 48] claim 40, wherein the at least one or a plurality of first identifying linker oligonucleotides comprises a recognition motif for a site-specific nicking endonuclease, wherein the recognition motif is correlated to the sequence of nucleotides in the single stranded first, unique nucleotide sequence.

58 (amended). Method of [any of claims 55 to 57] claim 55 and comprising the further step of contacting the at least one or a plurality of chimeric polynucleotide tags with a site-specific restriction endonuclease capable of recognising a recognition motif of the chimeric polynucleotide tag fragment and cleaving said fragment.

59 (amended). Method of [any of claims 50 to 58] claim 50, wherein the plurality or subset of second identifying linker oligonucleotides is attached to a solid support.